Page No.	Date of	
6.2.i		

HÆMATOLOGICAL TECHNIQUE

HÆMATOLOGICAL TECHNIQUE

for Medical Laboratory Technicians and Medical Students

by

E. M. DARMADY
M.A., M.D. (Camb.), F.R.C.P.

Senior Pathologist, Portsmouth and Isle of Wight Area Pathological Service, formerly Assistant Pathologist, Salisbury General Infirmary and Wiltshire County Council. Pathological Specialist R.A.F.V.R., Demonstrator of Pathology, St. Bartholomew's Hospital, London

and

S. G. T. DAVENPORT
F.I.M.L.T.

Chief Technician, Portsmouth and Isle of Wight Area Pathological Service

With four coloured plates
and 23 text figures

LONDON
J. & A. CHURCHILL LTD.
104, GLOUCESTER PLACE, W.1
1954

Printed in Great Britain

PREFACE

During the past ten years the department of Hæmatology has become a distinct and important branch of every hospital laboratory. At the same time the medical laboratory technicians have emerged from their merely passive role as cleaners of more specialised glassware, to become integral members of a team carrying out specialized hæmatological techniques, which are frequently of the utmost importance to the patient.

In taking up our appointments we were immediately faced with the responsibility of training a large number of young persons anxious to take up this branch of laboratory work, and with the ready help of the Municipal College, Portsmouth, were able to start classes. In searching for suitable books for instructors, we were impressed by the comparative lack of suitable material, and with the experience gained over the past four or five years, we have tried in this book to repair this omission. We have in particular stressed the technical side of the work, and have given alternative methods where practicable. We have also attempted to cover the hæmatological section of the Syllabus of the Institute of Medical Laboratory Technology. We further felt that no technician can give of his best without realising the theoretical purpose of his work, and we have therefore given a brief explanation of the fundamental mechanisms behind the disease process. We have thought it important that the technician should realise the inherent errors of his technique and at the same time should be able to appraise his observations critically, and therefore have included a chapter on Medical Statistics. We have also included a chapter on malaria, but as this is an important section in the Syllabus for the examination of Parasitology technique in the Institute of Medical Laboratory Technology, we have not felt it necessary to cover this subject extensively in a book concerned primarily with hæmatology. Memories of student days as a pathological clerk and junior demonstrator of pathology bring back the difficulty of attempting new and basic techniques, the majority of which were obtained from slightly more senior colleagues whose imaginations were more gifted than their knowledge of hæmatology. We therefore feel that this book may also be of help to the medical student and potential pathologist.

We could not, of course, attempt the writing of this book without expressing our indebtedness to Sir Lionel Whitby and Dr. C. J. C. Britton for allowing us to make use of ideas based on their book "Disorders of the Blood" Plates I and II, and Figs. 2 and 22. We are grateful to Dr. Rosemary Biggs and Dr. R. G. MacFarlane for advice on our chapter on Coagulation of Blood; also to Professor D. F. Cappell and Dr. E. H. Hutchinson for help concerning the histological preparation of Sternal Marrow and permission to quote from their article on the subject. We would also thank Dr. J. C. Turner of the

Presbyterian Hospital, New York, who was good enough to obtain specially and bring with him to this country a sample of blood from a case of Sickle Cell Anæmia, on which the coloured drawing Plate IV is based. We are grateful to Dr. R. I. S. Bayliss for allowing permission to modify the drawings of the Hæmocytometers and to Dr. N. M. Wintrobe for allowing us to reproduce the normogram Fig. 12, and Sedimentation Rate Chart Fig. 23, to Professor E. J. King for Fig. 11, and at the same time we would like to thank Dr. G. A. Harrison for Fig. 14, Dr. J. V. Dacie for Fig. 16, Colonel H. E. Shortt for Fig. 22. We are also indebted to Sir Cecil Wakeley, late P.R.C.S. Editor of Faber's Medical Dictionary for some of the definitions set out in the Glossary of Terms.

We are particularly indebted to Mr. L. F. Isherwood, F.I.M.L.T., Senior Technician in the Hæmatology Department for the four coloured plates and Fig. 20, and to Mr. K. Tyler, A.I.M.L.T., of Southampton, for drawing Figs. 1, 3, 4, 5, 6, 7, 8, 9, 10, 13, 15, 18 and 19. We are also grateful to Dr. S. C. Dobson and Dr. Rosemary Inee who were good enough to read and criticize the proofs. We would also like to thank Miss E. M. Hibbert for the preparation and typing of the manuscript.

Finally we would like to pay particular tribute to Mr. A. W. N. Addison, Chairman of the Portsmouth Group Hospital Management Committee, and to Mr. J. R. C. Miller, Chairman, and the members of the Portsmouth and Isle of Wight Area Pathological Board, who by their foresight and pioneer work in forming a unified service to cover five Management Committees enabled the junior and student technicians to spend a specified time in the hæmatological department learning and practising the more specialized techniques instead of being confined to laboratories of limited and more generalized outlook. To them we are also grateful for their continued support of us, the helpful way in which each new problem is solved, and we feel sure that by such action the patient is ultimately benefited.

E. M. Darmady.
S. G. T. Davenport.

Portsmouth.

CONTENTS

CHAPTER I

BLOOD FORMATION

1. Introduction
2. Site of Blood Formation
   (a) Mesoblastic phase; (b) Hepatic phase; (c) Myeloid phase
3. Blood Formation in the Adult
4. Theories of Blood Formation
   (a) Monophyletic theory; (b) Polyphyletic theory

Perhaps the most controversial aspect of hæmatology is the subject of blood formation, for although the various sites of blood formation are known, agreement has not yet been reached as to the mode of origin and the relationship of the different blood cells. The difficulty has arisen partly because observations have had to be made on both human and animal embryos, and partly because their appearance may vary if the cells have been fixed, or are examined in a wet preparation. The exact identification of the cells has therefore become the subject of speculation and a number of theories have been put forward. These theories will be discussed later in the chapter. Meanwhile, it is proposed to discuss the site of blood formation.

Blood is formed from the primitive connective tissue or mesenchyme and three different phases occur which gradually merge one into the other.

THE SITE OF BLOOD FORMATION

The Mesoblastic phase. Blood is formed early in the life of the embryo, for it has been observed in the yolk sac of a chick as early as the third day of its embryonic life. The site at which it is formed is known as the area vasculosa and consists of a number of small "blood islands". This is also thought to be true of the human. Soon the mesoderm of the area vasculosa differentiates into two layers forming a tube. The outer layer forms the primitive blood vessels, the inner layer the primitive endothelial cells, which later become detached and develop into the primitive blood cells. They have large nuclei composed of a loose chromatin network and have a relatively scanty cytoplasm. These cells are known by a number of names, for example, hæmocytoblast, megaloblast of Ehrlich, hæmotogone, hæmohistioblast, etc. As development proceeds, the primitive erythroblast is formed from the hæmocytoblast which projects into the lumen of the blood vessel and begins to develop hæmoglobin and to form nucleated red cells. Soon, however, these cells slowly

disappear and are replaced by similar but smaller cells known as definitive erythroblasts. Such cells are able to give rise to the ordinary red cell. The formation of erythroid cells from within the primitive blood vessels continues in the human up to about nine weeks of embryonic life. In the meantime the hepatic phase of blood formation has started.

The Hepatic phase. This phase is thought to begin about the sixth week of embryonic life. Nests of definitive erythroblasts start to appear in the liver, and seem to be derived from the layer of undifferentiated cells known as the mesenchyme, which separates the liver cells. The erythroblastic or erythrocyte series are formed inside the blood vessels (intravascularly). Although the bulk of the red cells are produced in the liver, small quantities are also made in the spleen and thymus until about the fifth month. At about the second month the granular cells appear in increasing numbers reaching their peak about the fourth month. These are produced extravascularly, and have to find their way into the blood through the capillary walls. Lymphocytes are produced chiefly in lymphatic tissue, spleen, thymus and lymph nodes and this persists in adult life.

The liver continues to make red and granular cells in diminishing numbers up to the birth of the fœtus, and at about the fifth month of uterine life the marrow starts to take over these functions, commencing the myeloid phase.

The Myeloid phase. At first the marrow is concerned only with formation of granular cells, but later with red cells as well, whilst the liver mesenchymal cells become quiescent. These liver cells, together with mesenchymal cells found elsewhere in the body, are converted to what is known as the reticulo-endothelial system; and the lining cells take over a new function. These, too, have been given a number of new names, for example, macrophage, clasmatocyte, hæmohistioblast, etc. They closely resemble large monocytes and can occasionally be found in the blood streams. By about the time of birth, the marrow has completely taken over the production of red and granular cells, but in the event of sudden crisis such as hæmorrhage, and in some diseases, the liver can revert to its original function of blood cell formation.

BLOOD FORMATION IN THE ADULT

At the time of birth all the fœtal bones show evidence of marrow activity. With age, some of the marrow is replaced by fat, and only certain bones, for example the sternum, the vertebræ, the ribs and the ilium, continue to make blood, while the lymphocytes and monocytes are formed in the reticulo-endothelial system, particularly in the lymph nodes and spleen.

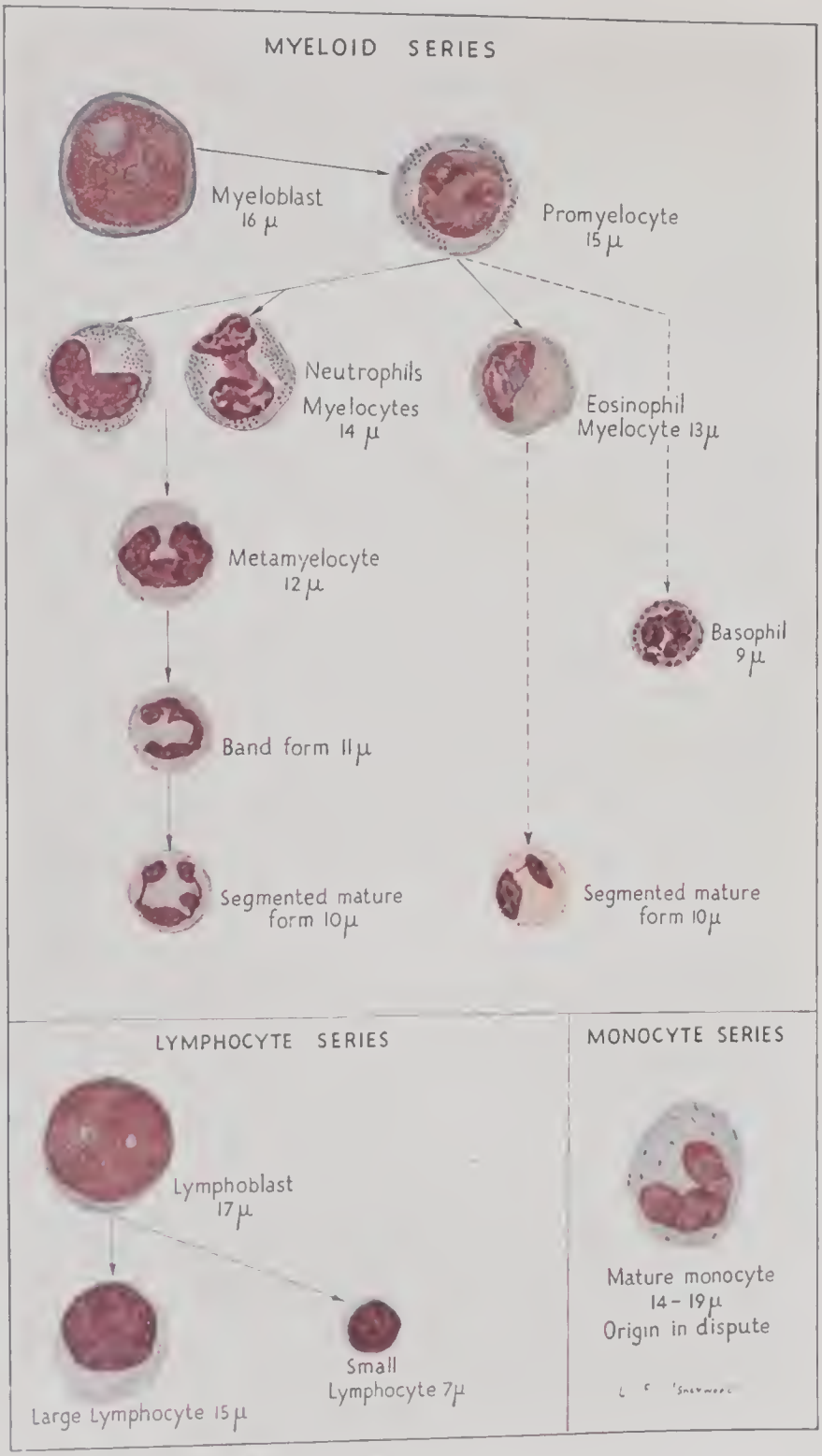
THEORIES OF BLOOD FORMATION

As has already been pointed out, doubt still exists as to the relationship of the various series of blood cells. The argument centres round the problem of whether a single precursor cell can give rise to all types

of blood cells (the monophyletic theory) or whether there are a number of specialized precursors (similar in appearance) which can give rise only to their own particular specialized series (the polyphyletic theory). From a clinical point of view the latter theory would appear more acceptable, since the increase of any one particular group may be achieved quickly, as for example in the leucocytosis of sepsis. It is not possible in the space available to enter more fully into the merits of each theory, and it is therefore better to consider the matter as still "sub-judice".

In Chapters II and III the sequence of events will be described with particular reference to the identification of cells in each series.

PLATE I



The cells are drawn to scale 1 mm. = 1 μ.

To face page 5]

MYELOBLAST.

Note: 1. The size of the cell, and space occupied by nucleus.
2. The presence of at least two nucleoli, and remnants of others.
3. Auer bodies.

PROMYELOCYTE.

Note: 1. The reduction in size and the shrinkage of the nucleus.
2. Absence of nucleoli, but some remnants.
3. Presence of cytoplasmic granules.

MYELOCYTE.

Note: 1. The shape of nucleus which is now indented and is bean shaped.
2. The space occupied by the nucleus.
3. Number and staining reaction of the cytoplasmic granules.

EOSINOPHIL MYELOCYTE.

Note: 1. The granules are coarser than those of myelocytes and fill all available cytoplasm, their colour is characteristic.

METAMYELOCYTE.

Note: 1. The "Dumb Bell" shaped nucleus.

NEUTROPHIL POLYMORPHONUCLEAR.

Note: 1. Size of the cell in comparison with its precursor.
2. Number of shaped lobes of the nucleus.
3. The thin strands of chromatin joining the lobes.
4. Intense staining reaction of the reticular network of nuclear lobes.

EOSINOPHIL POLYMORPHONUCLEAR.

Note: 1. Again the size; coarse and large cytoplasmic granules occupy the whole of the cell. Their colour is again distinctive.
2. The nucleus is bilobed.

BASOPHIL POLYMORPHONUCLEAR.

Note: 1. Reduction in cell size as compared with neutrophil or eosinophil myelocyte.
2. The presence of relatively few but distinctive basophilic granules.

LYMPHOBLAST.

Note: 1. The similarity of the general diameter to myeloblast. The size of the cell is usually smaller.
2. The absence of Auer bodies.
3. The coarser reticular pattern of the nucleus.
4. The number of nucleoli not more than two.

LARGE LYMPHOCYTE.

Note: 1. The dense deeply staining chromatin and almost circular nucleus.
2. The absence of nucleoli in the nucleus.
3. The pale cytoplasm with a few azurophil red granules.
4. The size of the cytoplasm.

SMALL LYMPHOCYTE.

Note: 1. The homogenous staining properties of the nucleus.
2. Comparative absence of cytoplasm and granules.

MONOCYTE.

Note: 1. The size of the cell 14–19 μ.
2. The nucleus occupies about half the available space.
3. The variability and shape of the nucleus which are waisted.
4. The presence of Auer bodies.